CONSTITUTIVE AND INDUCIBLE PECTINOLYTIC ENZYMES FROM *ASPERGILLUS FLAVIPES* FP-500 AND THEIR MODULATION BY PH AND CARBON SOURCE

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ABSTRACT

Growth and enzymes production by *Aspergillus flavipes* FP-500 were evaluated on pectin, polygalacturonic acid, galacturonic acid, arabinose, rhamnose, xylose, glycerol and glucose at different initial pH values. We found that the strain produced exopectinases, endopectinases and pectin lyases. Exopectinases and pectin lyase were found to be produced at basal levels as constitutive enzymes and their production was modulated by the available carbon source and pH of culture medium and stimulated by the presence of inducer in the culture medium. Endo-pectinase was basically inducible and was only produced when pectin was used as carbon source. Our results suggest that pectinases in *A. flavipes* FP-500 are produced in a concerted way. The first enzyme to be produced was exopectinase followed by Pectin Lyase and Endo-pectinase.

Keywords: *Aspergillus flavipes*, pectinolytic enzymes, constitutive enzymes, inducible enzymes, pectin.

INTRODUCTION

Pectin is composed of distinct polysaccharides fractions, such as homogalacturonan (HG), xylogalacturonan (XGA), rhamnogalacturonan I (RGI) and rhamnogalacturonan II (RGII). HG is known as the "smooth region" of pectin, it is a linear polymer composed of 1,4-linked α-D-galacturonic acid residues. Some of these residues can be, to different extent, methyl-esterified at the carboxylic acid group (C6 position) and/or can carry acetyl groups on C-2 and C-3. In XGA β-D-xylose residues are β-(1,3)-linked to the galacturonyl residue of the HG backbone. RGI is composed of alternating α-(1,2)-linked rhamnosyl and α-(1-4)-linked galactosyluronic acid residues. The rhamnosyl residues are branched with O-4 attached neutral sugars side chains that can vary from a single galactose residue up to polymeric chains of glycosyl residues composed of arabinose (arabinan side chains) and/or arabinose and galactose residues (arabinogalactan side chains). RGII is comprised of a backbone of approximately nine α-(1,4)-linked D-galactosyluronic acid residues that carry four side chains. Which consist of a number of rare sugars such as apiose, fucose, aceric acid and other. XGA, RGI and RGII are part of the branched region of pectin also known as "hairy region" (5,21).

The complex and heterogeneous structure of pectin has implications regarding the enzymes involved in its degradation and requires the presence of many different enzymatic activities. The main-chain-degrading enzymes are classified into esterases and depolymerases. The latter are divided into hydrolases (exo and endo types) and lyases (pectin and pectate). On the one hand, hydrolases (EC 3.2.1.15, EC 3.2.1.67 and EC 3.2.1.82) incorporate a water molecule via general acid catalysis during the cleavage of the glycosidic bond between the two saccharide units. On the other, lyases (EC 4.2.2.2, EC 4.2.2.9 and EC 4.2.2.10) cleave the glycosidic bond via a β-elimination reaction that removes a proton, resulting in an unsaturated bond between C-4 and C-5 of the saccharide unit at the nonreducing end (5,6).

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Pectin-degrading enzymes are widely used in a number of industries, including food processing, textile industry, wastewater treatment, animal feed, pulp and paper industry, among others (9,17). In addition, the understanding of the regulation process of the production of polygalacturonases will contribute not only to improve enzyme production, but also to get insights in the molecular dialogue between the host and the pathogen, during the microbial invasion of plant cell wall (7,11,22).

Pectin-degrading enzymes are produced by many microorganisms. Among them, *Aspergillus* species are widely used to produce a large number of enzymes from a great variety of carbon sources (24). Since *Aspergillus*, as is the case of other microorganisms, is not able to import polysaccharides, it has been proposed that mono or oligosaccharides released from the polymers at early stages of fungal growth, actually trigger expression of the degrading enzymes (13). Moreover, it is generally accepted that in *Aspergillus niger*, D-galacturonic acid or a metabolite derived from it is acting as an inducer of at least 12 pectinolytic genes (3). However, there are other subsets of pectinolytic genes responding to the presence of L-arabinose, L-rhamnose and ferulic acid (3). Pectinase expression is closely related to the carbon source available (2,18,26). Evidence for pH-dependent expression of pectinolytic genes has also been pointed out for some *Aspergillus* strains (5,20,22).

The aim of this study was to elucidate the constitutive or inducible nature of pectin degrading enzymes produced by *Asperigillus flavipes* FP-500 and to evaluate the effect of initial pH of culture medium on growth and pectinase production.

MATERIALS AND METHODS

Microorganism

The microorganism used in this work was the white fungus *Aspergillus flavipes* FP-500, which produces extracellular pectinolytic activities at 37ºC. The microorganism was maintained and propagated on potato dextrose agar plates.

Preparation of inoculum

The inoculum for either flasks or fermentor was prepared by growing the strain on PDA plates during 3 days at 37ºC. After this time spores were harvested by addition of 10 ml of saline-tween solution (NaCl, 0.9% and Tween 80, 0.01%) per plate. The spore suspension was counted in a counting chamber microscopic cell (American Optical, Inc. USA) and diluted with the same solution so that a final concentration of 1×10^6 spores ml^{-1} of culture medium was reached.

Media and fermentation conditions

For the production of pectinolytic activity, shake flasks and bioreactor experiments were conducted. Firstly, for shake flasks experiments 500 ml Erlenmeyer flaks were used, each one containing 100 ml of culture medium, and were agitated at 200 rpm in a reciprocant incubator shaker (Newbrunswick Sci. Co., USA) maintained at 37ºC. Secondly, bioreactor experiments were conducted in a Bioflo 110 bioreactor (Newbrunswick Sci. Co., USA) filled with 5.0 L of culture medium. Sterile air was supplied at a rate 0.5 vvm and the agitation speed was 200 rpm. Temperature was maintained at 37ºC.

Basal medium used contained (g l^{-1}): K_2HPO_4, 2; KH_2PO_4, 2; and $(NH_4)_2SO_4$, 5 and was supplemented with the chosen carbon source. Citrus pectin (P), polygalacturonic acid (PGA), galacturonic acid(GalA), arabinose (Ara), rhamnose(Rha), xylose (Xyl), glucose (Glc) and glycerol (Gly) (Sigma-Aldrich, Co., USA) all at 10 gl^{-1} were used as carbon sources. When simple sugars or glycerol were used 0.1% of yeast extract was added. Sterilization was carried out at 121ºC and 15 psi for 20 min. The initial pH of the medium was adjusted to 3.5, 4.2 and 5.0 with 2M NaOH or H_2SO_4.

Cell growth

Cell growth was measured as dry weight (DW). After filtering the sample through a Millipore membrane (pore size 5.0 μm, Millipore Mexico, S.A. de C.V.), previously dried to constant weight, the retained cell mass was dried at 80ºC until constant weight was reached.

Assays for pectinolytic activity

Samples were taken from flasks or fermentor at various times during fermentation and were immediately filtered through Millipore membrane. Pectinolytic activity of these cell-free samples was determined. Exo-pectinolytic (Exo-PG)activity was measured by quantifying the concentration of reducing groups which had been liberated after incubation (45ºC, 20 min) with 1% pectin (w/v) in 50 mM acetate buffer at pH 5.0. One Unit of exo-pectinolytic activity was defined as the amount of enzyme that catalyzes the formation of 1 μmol of galaturonic acid under assay conditions (25). For endopolygalacturonase (Endo-PG), by measuring the relative change in viscosity of 1% pectin in 16 mM acetate buffer added with 41 mM NaCl at 30ºC in a Canon Fenske 200 viscosimeter. One Unit of endopolygalacturonase activity was defined as the amount of enzyme that reduced the viscosity of 10 ml of pectin by 50% in 10 min at pH 4.2 under the assay conditions (25). Pectin-lyase (PL) activity was measured by monitoring the increase in absorbance at 235 nm brought about by the appearance of the double bond between carbon atoms 4 and 5 of galacturonic acid. We used a modification of the technique proposed by Delgado *et al.* (6). Briefly, the reaction mixture contained 1.0 ml of 1% pectin in 50 mM TRIS-acetate buffer pH 8.8, and 0.2 ml of cell-free sample. Incubation was carried out at 40ºC for 1 h, after this time 0.2 ml aliquot was taken from the reaction mixture and added to a test tube containing 1.8 ml of 10 mM HCl to stop the reaction. One Unit of pectin lyase activity was defined as the amount of enzyme that causes

an increase of 0.1 units of the absorbance at 235 nm, under assay conditions.

Reducing sugars concentration

Reducing sugars were analyzed using 3,5-dinitrosalicilic acid (DNS) method (15) with the appropriate sugar as the reference standard.

Statistical analysis

Treatment effects with triplicated assays were compared by least significant difference (LSD) methodology (16). Thus, statistically significant differences between average values were determined with the SAS® software at a confidence level $\alpha = 0.05$.

Pectinases productivity calculation

Volumetric production rates for biomass (q_x) and enzymatic activities (q_{pE}) were calculated by:

$$q_x = \frac{Produced\ biomass\ (X)}{time\ period} = \frac{X_2 - X_1}{t_2 - t_1}\ [=]\ \frac{mg\ dry\ biomass}{ml.h}$$

and,

$$q_{xE} = \frac{Produced\ enzymatic\ activity\ (E)}{time\ period} = \frac{E_2 - E_1}{t_2 - t_1}\ [=]\ \frac{U}{ml.h}$$

The $q_{pE\ max}$ and the corresponding q_x were used to obtain the specific enzyme productivity through:

$$P_{E/X} = \frac{q_{pE,max}}{q_x}\ [=]\ \frac{U}{mg\ dry\ biomass}$$

RESULTS

Pectinases produced by *Aspergillus flavipes* FP-500 growing on pectin at different initial pH conditions

Initial pH was evaluated on shake flasks and at bioreactor level. As it can be seen in Fig.1, when shake flasks experiments were conducted, cell growth of *A. flavipes* FP-500 on pectin reached a maximum of 4.5 mg ml^{-1} at an initial pH of 5.0. Lower growth, around 3 mg ml^{-1}, was attained at the other pH values (Fig. 1A).

Despite the lower cell growth attained at pH 3.5, Exo-PG production was higher in relation to other pH values tested and in fact, the difference was quite clear since early stages of fermentation (Fig. 1B). At 24 h when the initial pH was 3.5 Exo-PG activity represented around 3.8 times higher than that obtained at pH 4.2 (Fig. 1B). A similar result was obtained for Endo-PG production, which was also higher at pH 3.5 (Fig. 1C). However, the difference at the distinct pH values was more evident at later fermentation time. Quite the opposite was observed for PL production. During the first 48 h of culture, the
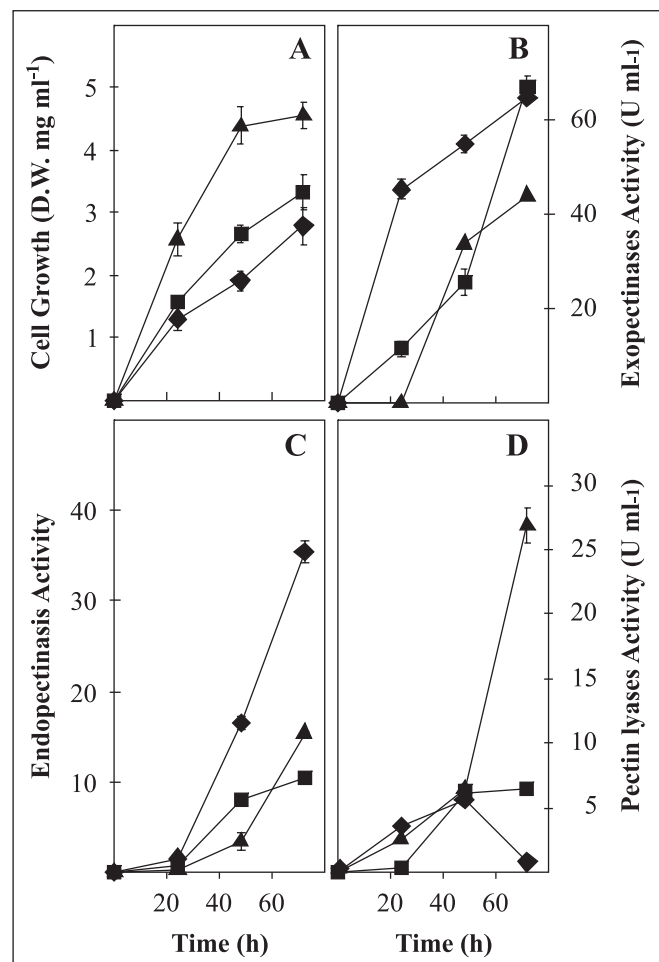

Figure 1. Growth (A), Exo-PG (B), Endo-PG (C) and PL (D) activities produced by *Aspergillus flavipes* FP-500 growing in shaken flasks with 10 g l^{-1} of pectin at initial pH of 3.5 (◆), 4.2 (■) and 5.0 (▲).

activity remained similar irrespective to the pH of culture medium (Fig. 1D). However, at a later stage (72 h) lyase activity was around 4 times higher when the pH was 5.0 (Fig. 1D).

According to shake flask experiments, the initial pH of the medium and its evolution affected the pectinases produced by *A. flavipes* FP-500. At 5 L fermentation level it was possible to get more samples during fermentation, so that a better picture of the evolution of the fermentation was obtained. As can be seen from Fig. 2, at early stages of fermentation pectin was degraded and its products accumulated (Fig. 2A). A maximum was attained between 24 and 36 hours after which a reduction in their concentration was observed. At this level the cell growth evolution was slower than in shaken flask experiments, although a maximum growth was attained at the same pH value on both systems (data not shown).
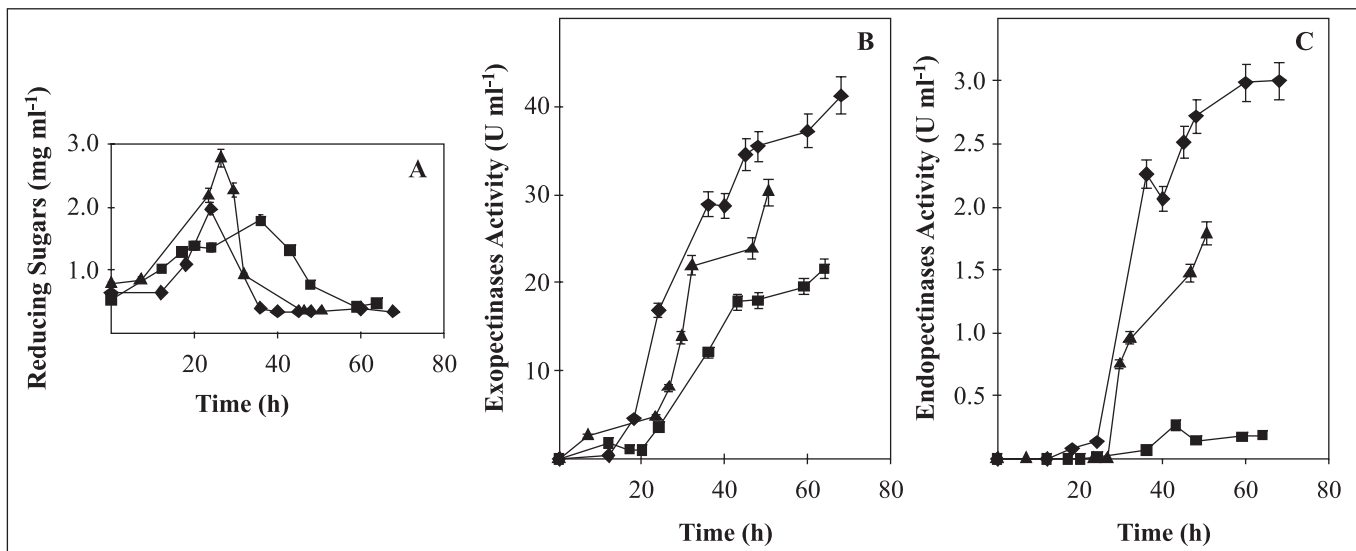
Figure 2. Reducing sugar concentration (A), Exo-PG (B) and Endo-PG activity (C) produced by *Aspergillus flavipes* FP-500 growing in a 5.0 L bioreactor with 10 g l⁻¹ of pectin, at initial pH of 3.5 (◆), 4.2 (■) and 5.0 (▲).

As a consequence of the reduction on the reducing sugars' concentration, an increase in Exo-PG and Endo-PG activity was observed (Fig. 2A-C). In these experiments, maximum production of both enzymes was attained at 3.5 pH (Fig. 2B and 2C), in the same way as in the results obtained in shaken flask experiments.

In addition, when the strain grows on pectin, there is subtle change in pH during the first 24 hours (Fig. 3A). However, after a media acidification in between 24-30 h, culture pH increased to reach values higher than the initial (Fig. 3A). Exo-PG showed to be modulated by pH of culture medium. However, a more dramatic influence on Endo-PG activity was observed (Fig. 2C) and PL (Fig. 3B). Surprisingly pectin-lyase maximum production was attained at pH 3.5. This later result was not expected, since on shake flask experiments PL activity was found to be higher at pH 5.

Pectinase production on different carbon sources and pH values

Growth and production of pectinase by *Aspergillus flavipes* FP-500 with different carbon sources at three pH values were carried out in shaken flask cultures. Results are presented in Tables 1-3. Exo-PG was produced practically on all carbon sources, including those not structurally related to pectin, such as glycerol. Moreover, low Exo-PG productivity was measured also in media containing glucose as the only carbon source (Table 1). This fact suggests that Exo-PG could be constitutive in *A. flavipes* FP-500. It should be noted that there is an important difference in the production of Exo-PG with simple sugars and pectin, (i.e at pH 4.2, 3.03 U mg⁻¹ dry biomass and 63.82 U mg⁻¹ dry biomass, were obtained for xylose and pectin, respectively), indicating that the production of the enzyme is also stimulated by the presence of inducers.

Even though, initial pH had not a clear trend with simple sugars, it seem that the most favorable initial pH was 3.5, except

Table 1. Exo-PG productivity of *A. flavipes* FP-500 in submerged culture using different carbon sources[1].

Carbon source	$P_{E/X}$ (U/mg dry biomass) Initial pH			
	3.5	4.2	5.0	*LSD**
Arabinose	1.50 ± 0.042	7.37 ± 0.21	3.17 ± 0.092	*0.0338*
Galacturonic acid	7.85 ± 0.39	0.78 ± 0.04	5.10 ± 0.26	*0.5418*
Glucose	0.25 ± 0.042	0.52 ± 0.21	0.36 ± 0.092	*0.0392*
Glycerol	7.28 ± 0.360	5.5 ± 0.08	2.83 ± 0.14	*0.4595*
Pectin	34.95 ± 1.75	63.82 ± 3.2	18.68 ± 0.94	*4.3327*
Polygalacturonic acid	5.21 ± 0.26	24.54 ± 1.23	12.44 ± 0.62	*1.6150*
Rhamnose	1.09 ± 0.05	12.92 ± 0.65	1.59 ± 0.08	*0.7543*
Xylose	0.16 ± 0.008	3.03 ± 0.15	2.81 ± 0.14	*0.2385*
LSD†	*1.0578*	*2.0012*	*0.6742*	

[1] Experiments were conducted on shake flasks as described in Materials and Methods section; * Differences between data in the same row bigger than this LSD value are statistically significant; † Differences between data in the same column bigger than this LSD value are statistically significant.
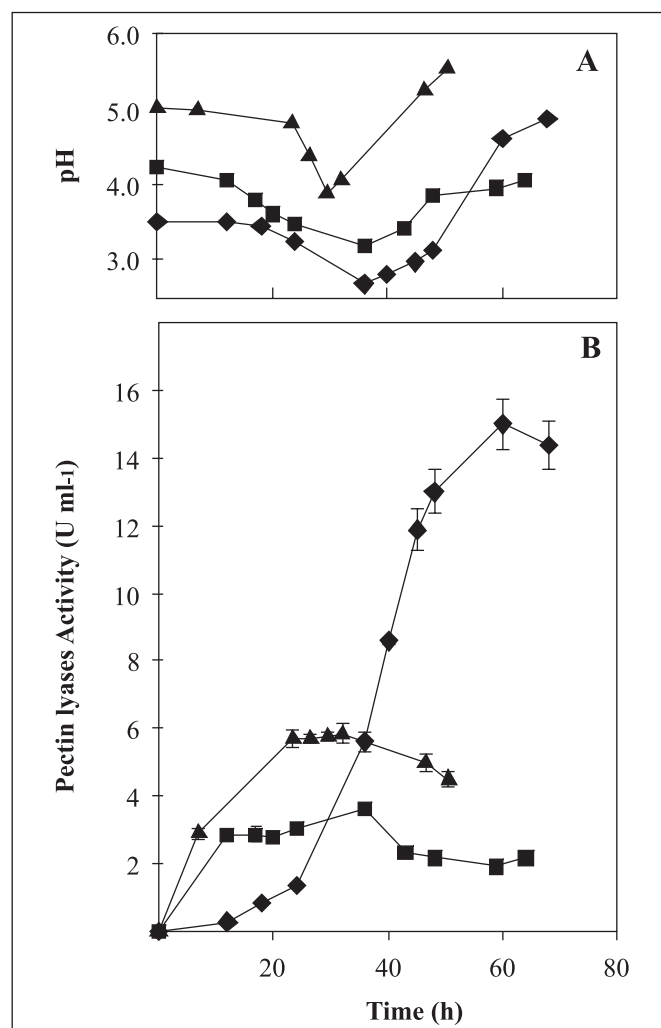
Figure 3. Evolution of pH (A) and PL activity (B) produced by *Aspergillus flavipes* FP-500 growing in a 5.0 L bioreactor with 10g l⁻¹ of pectin, at initial pH of 3.5 (◆), 4.2 (■) and 5.0 (▲).

for rhamnose where the maximum productivity was attained at pH 4.2 (Table 1).

Concerning Endo-PG, enzymatic activity produced with simple sugars as carbon source was barely observed on galacturonic acid (Table 2). With xylose, arabinose and glucose very low levels (<0.1 U ml⁻¹) of activity were found. However, these values are lower that the LSD, so it is not possible to make reliable a conclusion about these numbers, and in fact the activity produced with galacturonic acid is not conclusive at all. Endo-PG specific activity was significantly higher (24.7 U mg⁻¹) on pectin with an initial pH of 3.5. Surprisingly, polygalacturonic acid was not a good carbon source (Table 2). It also should be highlighted that Endo-PG are produced in relatively high levels in a late culture stage (~50 h), after an earlier Exo-PG action on pectin.

Pectin lyase activity was observed in almost all substrates (Table 3). The lower specific activity was obtained when *Aspergillus flavipes* FP-500 was grown on glycerol and xylose (Table 3). A remarkable fact is that PL are present in all carbon sources, suggesting that this enzyme could probably be of a constitutive nature.

DISCUSSION

Growth and pectinase production by *Aspergillus flavipes* FP-500 were affected by the initial pH of culture medium (Fig. 1). Higher Exo- and Endo-polygalacturonase activities were obtained at 3.5 (Fig. 1). According to our results, *A. flavipes* FP-500, produced in constitutive manner, basal levels of Exo-PG which starts to act on pectin, releasing reducing sugars to the medium. Afterwards, the reducing sugars produced trigger the massive production of Exo-PG. Therefore, it could be considered that these enzymes are also inducible to a certain level. The inducibility and constitutiveness of Exo-PG have been reported in other *Aspergillus* species (1,3,8). The fact that Exo-PG was produced at early stages of fermentation allows a fast appearing of degradation products from pectin and the concomitant growth of the fungus (Fig. 1-3). Pectin degradation at this stage also provides inducers for Endo-PG and PL (Fig. 1-3). This is supported by the fact that Exo-PG is produced to different extent in different carbon sources (Table 1). It is possible to notice a trend to produce this activity by the fungus at lower pH (i.e. 3.5) but the most important thing is that activity was present in all cases. It is interesting that exo-activity produced on GalA and Gly was higher than those observed on the other simple sugars tested. However, from our results Exo-PG is considered a constitutive enzyme whereas GalA is proposed as inducer. On the other hand, in relation to Gly which is not part of pectin structure, it has recently been reported that in the catabolic pathway of galacturonic acid in *Hypocrea jecorina* (*Trichoderma reesei*), glycerol is obtained in the last stages of this route as a product of galacturonic acid degradation (10) and also that these catabolic pathway is conserved in *A. niger*. This could explain the relatively high productivities obtained on this carbon source. Induction of pectinases by GalA has been demonstrated in another *Aspergillus* species (23). Also it is an accepted fact that pectin and glycerol activate the expression of pectinases encoding genes on *A. niger* (4). However, it can not be discarded that Exo-PG respond also to the presence of Ara and Rha (Table 1).

The production of Endo-PG correlates with the reduction in the concentration of reducing groups at later time during fermentation (Fig. 2A and 2C) indicating control by catabolic repression, as it has been reported for several *Aspergillus* endo-pectinases (19). Unlike exo- activity, Endo-PG was only produced on pectin (Table 2) and the high productivity was found using pectin as carbon source. It was expected that this enzyme was produced also on polygalacturonic acid and eventually on GalA,

but the activity produced on these carbon sources was negligible (Table 2). In both cases the productivity was lower than LSD, so is not possible to get reliable conclusions with this data. This is quite possible related to the evolution of the pH during culture. When pectin is used as carbon source the pH tend to decrease during the first hours of culture and to increase at later stages (Fig. 3). With glucose, xylose, arabinose, rhamnose and glycerol, the pH decreases, so that by the end of fermentation it reaches values between 2.2-2.8. However, with GalA and PGA, the pH behaved in the opposite way. The initial values in the culture medium tend to increase continuously reaching values of 6 to 7.5, even when the initial pH is 3.5. This behavior has also been observed with *Aspergillus flavus* NRRL-2687 and CECT-6541 (Gomez-Sanchez and Aguilar, unpublished results).

Apart from this, as shown in the pectin experiments (Fig. 1), while initial pH increased, Endo-PG production was lower. So, in the case of GalA and PGA the lack of production of Endo-PG surely was due to the evolution of the pH. These findings indicate that Endo-PG are truly inducible, and that pectin is the main inducer.

The lyases production with *A. flavipes* FP-500 occurred in practically all carbon sources with a trend to be produced at pH 5.0 (Table 3). Similar to Exo-PG, PL seems to be constitutive. The enzyme was produced at low level on different carbon sources but its production was stimulated by the presence of pectin and polygalacturonic acid. The constitutive expression of PL genes *pel*B, *pel*C and *pel*F has been reported in *A. niger* growing on a number of substrates, including glucose (3) and *Penicillium griseoroseu* was also reported to produce pectin lyases without any inducer (21). It is interesting to notice that this enzyme is only produced with PGA at pH 5.0, this fact reinforces the idea that the production of pectinases by this fungus is the result not only of initial pH, but also of the evolution of this factor during culture.

As we discussed above, firstly, when *A. flavipes* was grown on PGA the pH tends to increase in spite of the initial value, but only when initial pH was set to 5.0, pectin-lyase was produced.

Results obtained on bioreactor experiments seem to indicate an opposite behavior of the lyase production pattern in relation to pH (Fig. 3). In this experiment, higher activity was obtained at 3.5 (Fig. 3). Furthermore, the difference between the results in shaken flask and bioreactor experiments could be attributed to the difference in the availability of the dissolved oxygen in the medium. In addition, it has been reported that the capability of oxygen transfer in shaken flask, operated at conditions established in

Table 2. Endo-PG productivity of *A. flavipes* FP-500 in submerged culture using different carbon sources [1].

Carbon source	$P_{E/X}$ (U/mg dry biomass) Initial pH			
	3.5	4.2	5.0	*LSD**
Arabinose	0.13 ± 0.004	0.0	0.0	*0.0013*
Galacturonic acid	0.50 ± 0.025	0.0	0.0	*0.0500*
Glucose	0.052 ± 0.004	0.0	0.0	*0.005*
Glycerol	0.0	0.0	0.0	—
Pectin	24.7 ± 1.24	6.78 ± 0.34	1.67 ± 0.08	*1.4804*
Polygalacturonic acid	0.22 ± 0.011	0.0	0.37 ± 0.02	*0.0248*
Rhamnose	0.0	0.0	0.0	—
Xylose	0.0	0.02 ± 0.001	0.006 ± 0.0003	*0.0012*
LSD[†]	*0.7064*	*0.1938*	*0.0489*	

[1] Experiments were conducted on shake flasks as described in Materials and Methods section; * Differences between data in the same row bigger than this LSD value are statistically significant; [†] Differences between data in the same column bigger than this LSD value are statistically significant.

Table 3. PL productivity of *A. flavipes* FP-500 in submerged culture using different carbon sources [1].

Carbon source	$P_{E/X}$ (U/mg dry biomass) Initial pH			
	3.5	4.2	5.0	*LSD**
Arabinose	1.37 ± 0.04	1.80 ± 0.05	0.22 ± 0.006	*0.1046*
Galacturonic acid	1.18 ± 0.059	0.93 ± 0.0	1.68 ± 0.084	*0.1300*
Glucose	4.36 ± 0.04	4.37 ± 0.05	6.01 ± 0.006	*0.9896*
Glycerol	0.65 ± 0.0325	0.29 ± 0.015	0.18 ± 0.009	*0.0423*
Pectin	2.79 ± 0.14	5.42 ± 0.3	5.20 ± 0.11	*0.3738*
Polygalacturonic acid	0.0	0.0	17.71 ± 0.8	*1.7000*
Rhamnose	1.73 ± 0.09	0.87 ± 0.04	0.45 ± 0.023	*0.1147*
Xylose	0.49 ± 0.025	0.0	2.27 ± 0.11	*0.1339*
LSD[†]	*0.1643*	*0.2066*	*0.6903*	

[1] Experiments were conducted on shake flasks as described in Materials and Methods section; * Differences between data in the same row bigger than this LSD value are statistically significant; [†] Differences between data in the same column bigger than this LSD value are statistically significant.

our experiments, is enough to hold the fungal growth and pectinolytic enzyme production in non-limited oxygen conditions. In contrast, in the bioreactor experiments, oxygen was depleted quite soon, during the first 24h of culture oxygen, tension (TOD) was practically cero for pH 4.2 and 5.0 and it remained like that until the end of fermentation; whereas that

for pH 3.5 was around 20% at the same time. Therefore, cultures at pH 4.2 and 5.0 were carried out under limited oxygen conditions. It is well known that the genus *Aspergilli* produces different kinds of organic acids in non-limited oxygen condition (TOD>30%) such as citric acid, gluconic acid, fumaric acid and kojic acid with the concomitant acidification of the culture medium. When the oxygen transfer rate is the limiting step in the fermentation, there is not accumulation of dissolved oxygen causing an important lack of oxygen which changes the metabolism. This could explain both the increase in pH of fermentation broth, and the stop of production of PL at initial pH values of 4.2 and 5.0. Furthermore, the relative rate of PL synthesis during the first hours of fermentation in bioreactor experiments were 0.0233, 0.236 and 0.321 Uml-1h-1 for 3.5, 4.2 and 5.0 pH, respectively, indicating that the limiting step at this level was the dissolved oxygen tension (TOD) in the system.

CONCLUSIONS

Aspergillus flavipes FP-500 was able to grow and produce pectinase enzymes on different carbon sources at different initial pH. Exo-PG and PL were produced constitutively and were also stimulated by the presence of inducer in the culture medium. Pectin, polygalacturonic acid and galacturonic acid were found to be effectors of the induction. According to our results, GalA or a product of its catabolism could be proposed as the real inducer of pectinolityc enzymes in this strain. In contrast, Endo-PG was found to be basically inducible enzyme. Moreover, pectinases in *A. flavipes* FP-500 seem to be produced in a concerted way, according to the pH and to the available substrate. As a result, Exo-PG and PL were produced at the early stages of culture and Endo-PG at later.

Finally, as it has been discussed above, the production of these enzymes is not only related to the initial pH of culture medium, but also to it's evolution during fermentation.

ACKNOWLEGEMENTS

RESUMO

Enzimas pectinolíticas constitutivas e indutíveis de *Aspergillus flavipes* FP-500 e sua modulação pelo pH e fonte de carbono

Avaliou-se o crescimento e a produção de enzimas por *Aspergillus flavipes* FP-500 em pectina, ácido poligalacturônico, ácido galacturônico, arabinose, ramnose, xilose, glicerol e glicose, em diferentes valores de pH inicial. Verificamos que a cepa produziu exopectinases, endopectinases e pectina liases. Exopectinases e pectina liases foram produzidas em níveis basais como enzimas constitutivas e sua produção foi modulada pela fonte de carbono disponível e pelo pH do meio de cultura e estimulada pela presença de indutores no meio de cultura. Endopectinase foi indutível e produzida somente quando pectina foi utilizada como fonte de carbono. Nossos resultados sugerem que as pectinases de *A. flavipes* FP-500 são produzidas de forma planejada. A primeira enzima a ser produzida foi expopectinase, seguida por pectina liase e endopectinase.

Palavras-chave: *Aspergillus flavipes*, enzimas pectinolíticas, enzimas constitutivas, enzimas indutíveis, pectina.

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